Annls Soc. r. zool. Belg. — T. 113 (1983) — fasc. 2 — pp. 155-164 — Bruxelles 1983

(*Manuscript received on 18 August 1982*)

INTERNATIONAL STUDY ON *ARTEMIA* (1). XVII. ENERGY CONSUMPTION IN CYSTS AND EARLY LARVAL STAGES OF VARIOUS GEOGRAPHICAL STRAINS OF *ARTEMIA*

by

PAUL VANHAECKE, PATRICK LAVENS and PATRICK SORGELOOS (2)
Artemia Reference Center, State University of Ghent
J. Plateaustraat 22, B-9000 Ghent (Belgium)

SUMMARY

Variations in dry weight, caloric content and ash content during cyst hatching and early larval development have been studied for various geographical strains of *Artemia*. In general, decapsulated cysts contain 30 to 40 % more energy than freshly hatched nauplii; for Chaplin Lake and Buenos Aires *Artemia* this difference amounts to 57 %. Ash contents increase as decapsulated cysts hatch into instar I and molt into instar II-III nauplii. Over a 24 h larval developmental period individual dry weights and energy contents of the nauplii decrease with 16-34 % and 22-37 % respectively.

A small but significant correlation exists between the survival rate of starved nauplii and either the energy content of instar I and instar II-III nauplii or the proportional energy consumption during metabolism from decapsulated cysts to instar II-III nauplii.

The potential impact of these results on the use of *Artemia* in aquaculture hatcheries is discussed.

INTRODUCTION

Artemia only can ingest food from the second instar stage onwards (BENESCH, 1969). As a result their organic contents decrease from the restart of the cyst's metabolism through the hatching of the nauplius and into the instar II larval stage (VON HENTIG, 1971) ; *e.g.* in individual nauplii of San Francisco Bay (California) origin dry weight and energy contents decrease by 20 % respectively 27 % as they molt from the instar I into the instar II-III stage (BENIJTS *et al.*, 1976). As a result *Artemia* nauplii as food source in aquaculture hatcheries are most efficiently used as freshly-hatched nauplii. In practice, however, not much attention is paid to this potential economy in *Artemia* cyst uses ; *e.g.* techniques for cyst incubation and nauplii harvesting are usually not standardized, *Artemia* nauplii are sometimes stored for one or more days prior to be fed to the predator larvae, as *Artemia* nauplii are distributed only once a day, their retention time prior to being captured by the predator larvae often exceeds 24 h.

In addition more and more new *Artemia* cyst sources are used (SORGELOOS, 1980a) and nothing is known about the potential differences in terms of critical drops in energy contents during larval developments, or survival rates in culture tanks in unfed conditions.

(1) International interdisciplinary study on *Artemia* strains coordinated by the *Artemia* Reference Center, State University of Ghent, Belgium.
(2) Research Associate at the Belgian National Science Foundation.

In this regard, we have studied nauplius survival at two different temperatures as well as energy contents and dry weights of instar I and instar II-III larvae for the most commonly used *Artemia* cyst sources.

Since it has recently been shown that decapsulated cysts can be used as a direct food source for many aquaculture organisms (BRUGGEMAN *et al.*, 1980) which could lead to a further economy in the use of *Artemia cysts*, we have also analyzed the dry weight and energy contents of decapsulated cysts for the same geographical *Artemia* sources.

MATERIALS AND METHODS

Details on the geographical origin of the *Artemia* strains studied are provided in Table I.

TABLE I

List of « Artemia » sources studied

Geographical source of cysts	batch number or year of harvest	mode of reproduction (B : bisexual; P : parthenogenetic)	abbreviation used
San Francisco Bay (Ca-USA)	nº 288-2596	B	SFB
San Pablo Bay (Ca-USA)	nº 1628	B	SPB
Macau (Brazil)	May 1978	B	MAC 1
	nº 871172	B	MAC 2
Barotac Nuevo (Philippines)	1978	B	PHIL
Chaplin Lake (Canada)	1978	B	CHA
Buenos Aires (Argentina)	1977	B	ARG
Bahia Salinas (Puerto Rico)	—	B	PR
Great Salt Lake (Ut-USA)	1977	B	GSL
Shark Bay (Australia)	nº 114	P	SB
Margherita di Savoia (Italy)	1977	P	MS
Tientsin (People's Republic of China)	1978	P	TIEN
Lavalduc (France)	1979	P	LAV

Cysts were incubated in natural seawater (35 ‰ salinity) at a temperature of $25^\circ \pm 0.5^\circ$ C and an illumination of 1,000 lux. A homogenous population of instar I nauplii was obtained by harvesting the nauplii when 90 % hatching was attained or, for the slow hatching strains, 8 to 10 h after the appearance of the first nauplii. The nauplii were separated from their hatching debris with a separator box (PERSOONE and SORGELOOS, 1972). Half of the nauplii was analyzed immediately, the other half was incubated in an Erlenmeyer flask, containing 1 l natural seawater at $25^\circ \pm 0.5^\circ$C that was aerated by gentle air-bubbling and illuminated at 1,000 lux. After 24 h incubation, the nauplii had molted into the instar II and some even in the instar III stage. They were separated from their exuviae and a few dead nauplii with the separator box and were analyzed.

Cysts that had been cleaned following the procedure of Sorgeloos *et al.* (1978) were decapsulated according to the technique of Bruggeman *et al.* (1980).

Dry-weight analyses of nauplii and cysts were carried out following the method described by Vanhaecke and Sorgeloos (1980). Ash weights were determined on dried samples that had been incinerated for 4 h at 550° C.

Energy contents were analyzed on six replicate samples of approximately 25 mg dry material per strain following the wet oxidation technique of Maciolec (1962).

The data obtained were analyzed for statistical significance with a one way analysis of variance (Model I ; Sokal and Rohlf, 1969). For comparisons among means, Duncan's Multiple Range test was used (Snedecor and Cochran, 1967).

The survival experiments were carried out in glass petri dishes. Per strain, three dishes each containing 10 freshly hatched nauplii in 10 ml filtered natural seawater were incubated in darkness at temperatures of 20° C and 30° C respectively. Two replicate survival tests were conducted for each *Artemia* strain. Once a day for the 20° C run and twice a day for the 30° C run, mortality was checked and dead larvae removed. LT 50 values were calculated according to the method of Litchfield (1949) and compared for statistical differences following the technique of Litchfield and Wilcoxon (1949).

RESULTS

From Table II it appears that the individual dry weights of decapsulated cysts vary considerably from one strain to another ; *i.e.* overall variation exceeding 100 %. No significant differences could be noted among batches from the same strain nor between cysts originating from the same parental material but produced at different localities, *e.g.* Macau, Barotac Nuevo and San Francisco Bay.

The variation in ash content between *Artemia* strains is rather small (3.78-5.36 %).

Differences in energy content between the strains studied are low, the maximal range being 9.3 %. Statistical analyses revealed, however, that the energy content of decapsulated cysts from the parthenogenetic strains of Margherita di Savoia, Tientsin and Lavalduc is significantly lower than for most other sources. Shark Bay *Artemia* make, however, an exception, being a parthenogenetic strain with a cyst energy-content similar to the values obtained for most bisexual strains.

As a result of the differences in individual dry weights, the individual caloric contents vary widely from one strain to another.

From the Tables III and IV it is clear that for the instar I respectively instar II-III nauplii variations in individual dry weight, ash content and energy content are similar to those of decapsulated cysts. As the embryos have developed from cyst to instar I and instar II or III, the ash contents have increased, whereas the individual dry weights and individual energy contents have dropped.

Statistical analysis of the survival data shown in Fig. 1 reveals a great similarity in response among *Artemia* from different geographical origin exposed to 20° C and 30° C. The nauplii of Chaplin Lake and Buenos Aires always die off earliest, whereas Shark Bay *Artemia* have the longest mean lethal time.

TABLE II

Individual dry weight, ash content, and energy content of decapsulated « Artemia » cysts from various geographical origin

Source of cysts	individual dry weight (in µg)	ash content (in % of dry weight)	energy content (in joules/g dry weight)	individual energy content (in joules)
San Francisco Bay	2.15	4.37	23 250	0.0500
San Pablo Bay	2.48	4.28	23 160	0.0574
Macau (May 1978)	2.25	4.31	22 650	0.0510
Macau (Batch 871172)	2.30	4.21	22 540	0.0523
Barotac Nuevo	2.21	4.09	23 370	0.0516
Chaplin Lake	3.05	5.36	23 010	0.0702
Buenos Aires	2.66	5.26	22 290	0,0593
Bahia Salinas	2.87	4.82	22 640	0,0650
Great Salt Lake	3.23	4.51	22 900	0,0740
Shark Bay	3.42	3.78	23 050	0.0788
Margherita di Savoia	4.51	4.49	21 380	0.0964
Tientsin	4.17	5.09	21 740	0.0907
Lavalduc	3.98	4.25	21 890	0.0871

TABLE III

Individual dry weight, ash content and energy content of instar I « Artemia » nauplii from various geographical origin

Source of cysts	individual dry weight (in µg)	ash content (in % of dry weight)	energy content (in joules/g dry weight)	individual energy content (in joules)
San Francisco Bay	1.63	6.33	22 480	0.0366
San Pablo Bay	1.92	6.17	22 330	0.0429
Macau (May 1978)	1.68	5.83	22 710	0.0381
Macau (Batch 871172)	1.74	5.88	22 520	0.0392
Barotac Nuevo	1.68	6.07	22 740	0,0382
Chaplin Lake	2.04	6.59	21 940	0.0446
Buenos Aires	1.72	6.32	22 020	0.0379
Bahia Salinas	2.10	5.51	22 390	0.0470
Great Salt Lake	2.42	5.69	22 350	0.0539
Shark Bay	2.47	5.28	22 330	0.0576
Margherita di Savoia	3.33	6.17	21 760	0.0725
Tientsin	3.09	6.63	22 050	0.0681
Lavalduc	3.08	6.03	21 760	0.0670

TABLE IV

Individual dry weight, ash content and energy content of instar II-III « Artemia » nauplii from various geographical origin

Source of cysts	individual dry weight (in µg)	ash content (in % of dry weight)	energy content (in joules/g dry weight)	individual energy content (in joules)
San Francisco Bay	1.25	10.35	21 520	0.0269
San Pablo Bay	1.36	10.20	21 110	0.0287
Macau (May 1978)	1.15	10.11	20 880	0.0240
Macau (Batch 871172)	1.21	10.21	21 120	0.0256
Barotac Nuevo	1.21	10.08	21 470	0.0260
Chaplin Lake	1.59	11.12	19 450	0.0311
Buenos Aires	1.13	9.75	20 370	0.0233
Bahia Salinas	1.68	9.87	21 810	0.0366
Great Salt Lake	1.59	9.61	21 310	0.0339
Shark Bay	2.07	8.54	21 680	0.0449
Margherita di Savoia	2.51	9.10	20 840	0.0523
Tientsin	2.37	9.84	19 940	0.0473
Lavalduc	2.20	9.33	20 150	0.0443

DISCUSSION

The data reported in this study clearly demonstrate that in *Artemia* both the individual weight and the energy content considerably drop as a result of the hatching metabolism (see Fig. 2). Since the energy contents expressed per gram organic weight do not change significantly as embryos develop into free-swimming nauplii, it is highly likely that the fat-protein-carbohydrate ratios remain approximately constant during this developmental period.

For most *Artemia* strains studied, the relative difference in weight and energy content between embryos and instar I nauplii is fairly constant within the range of 30-40 % ; Chaplin Lake and Buenos Aires *Artemia*, however, consume substantially more energy for hatching, relative differences in individual energy content amounting up to 57 %. It is not clear if this is related to the physico-chemistry of the biotopes, *e.g.* Chaplin Lake *Artemia* is the only sulphate strain studied here (Hammer, 1978), or the genotypical origin of the brine shrimp strain, *e.g.* Buenos Aires *Artemia* is the only representative of the *Artemia persimilis* sibling species whereas the other strains studied belong to either *Artemia franciscana* (7 sources) or *Artemia parthenogenetica* (4 sources) (Bowen *et al.*, 1978 ; Abreu-Grobois and Beardmore, 1980). The highest increases in hatching output at low salinity (5 ‰ instead of 35 ‰) being observed for precisely the same two strains (Vanhaecke and Sorgeloos, 1983) provides further support for the hypothesis of Sorgeloos (1980b) that the increase in hatchability at low salinities is related to energy.

In view of the data presented, it is clear that instead of freshly-hatched nauplii, the use of decapsulated cysts of *Artemia* as a direct food source can lead to a substan-

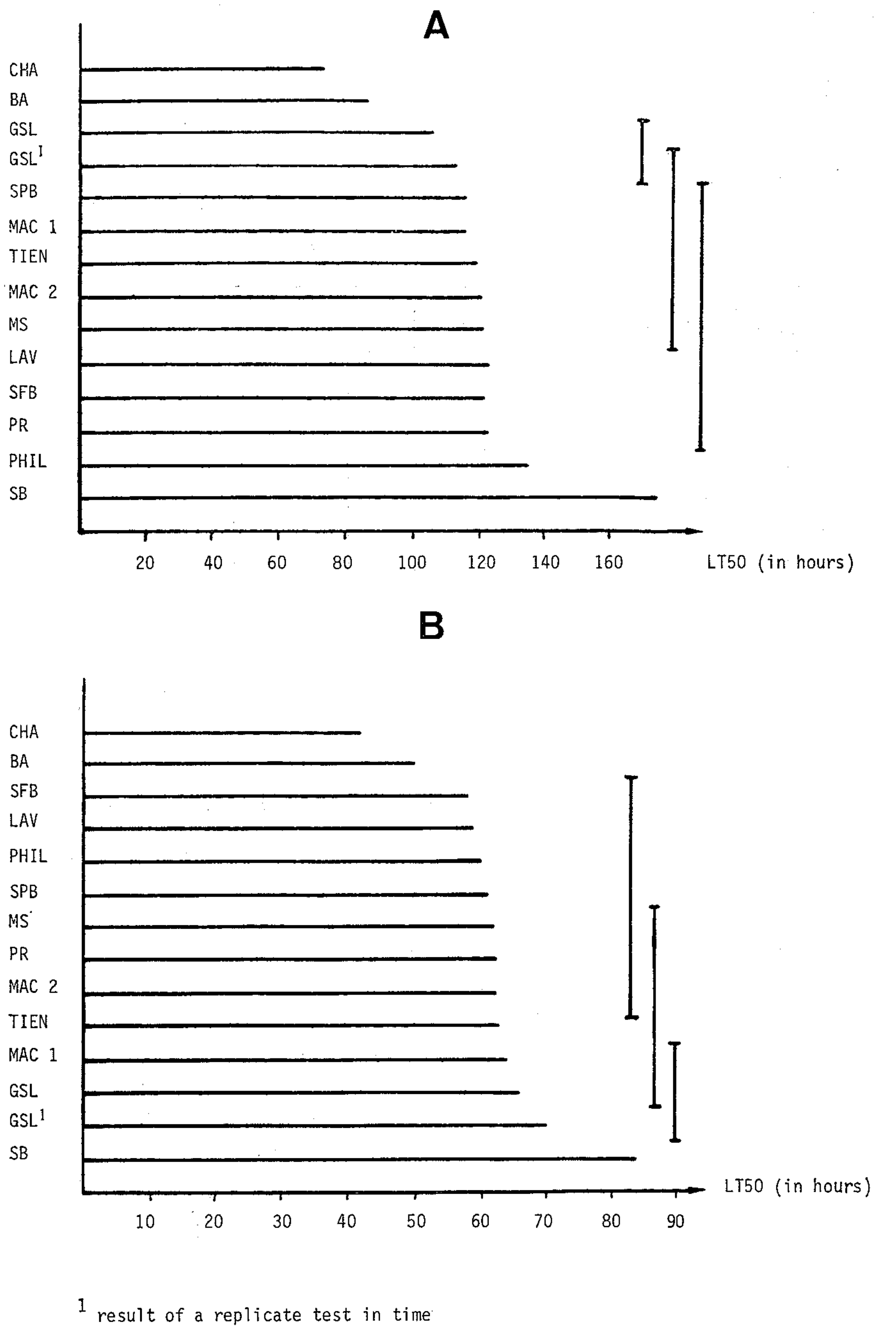

Fig. 1. — LT 50 values for unfed *Artemia* nauplii from different geographical origin incubated at 20° C (A) and 30° C (B). Strains connected by the same vertical line are not significantly different at the $P < 0.05$ level.

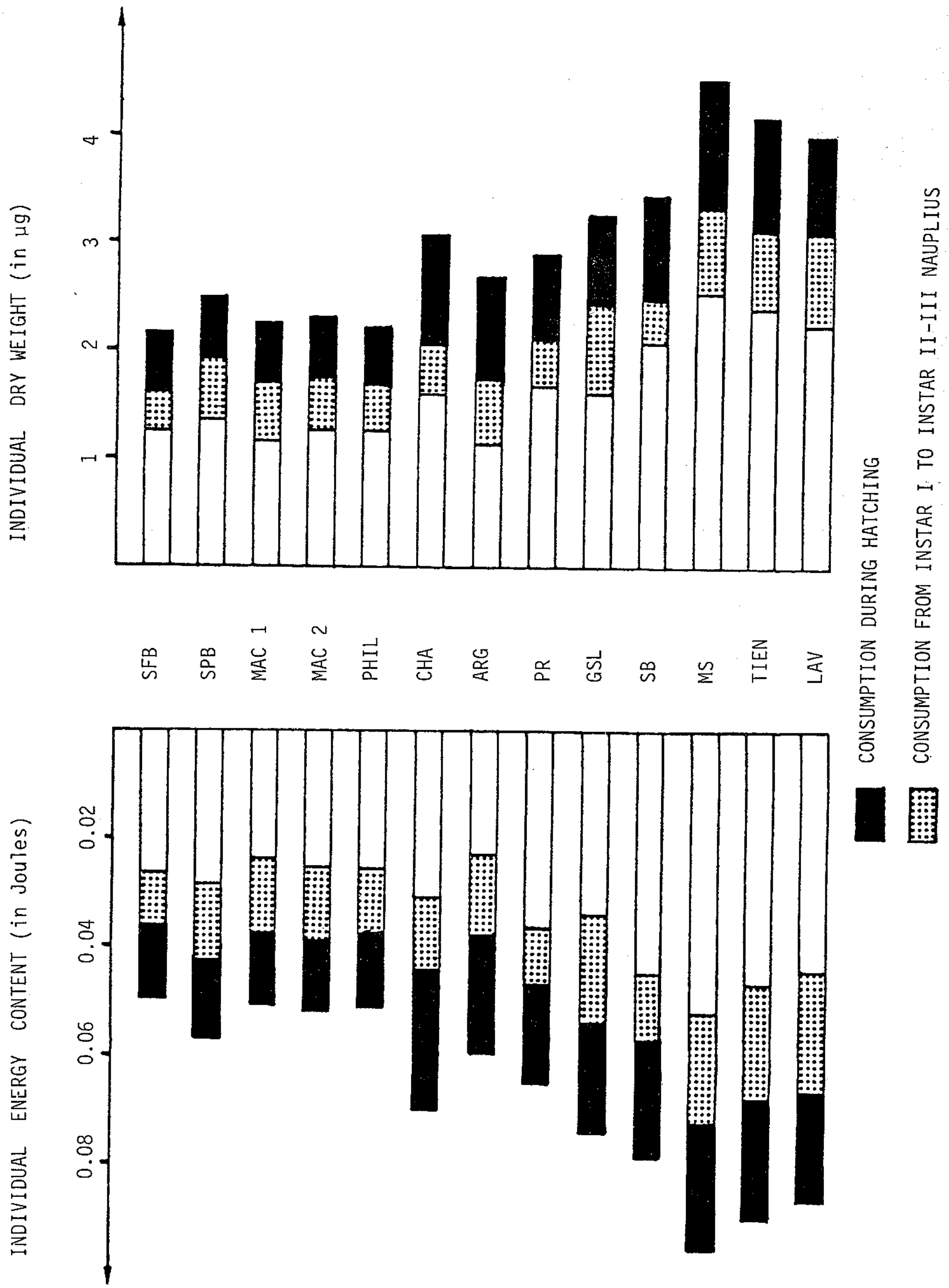

Fig. 2. — Comparison of the individual energy contents and individual dry weights of decapsulated cysts, instar I and instar II-III nauplii from different geographical strains of *Artemia*.

tial economy in aquaculture hatcheries. Decapsulated cysts not only contain 30 % to 40 % more energy as compared to freshly-hatched nauplii but their mean volume is on the average 30 % smaller (calculated from volumetric data by VANHAECKE and SORGELOOS, 1980 and VANHAECKE *et al.*, 1980). This means that d capsulated cysts can be offered to the fish and crustacean larvae at an earlier life stage. The promising possibilities in this regard have already been demonstrated for several predators (see BRUGGEMAN *et al.*, 1980).

Individual biomass and energy content further decrease as the free-swimming *Artemia* nauplii develop into instar II larvae (see Fig. 2). The data presented in this study for the San Francisco Bay strain are in accordance with the results of BENIJTS *et al.* (1976) : *e.g.* 23 respectively 20 % decrease in individual dry weight ; 26 respectively 27 % drop in individual energy content. Large variation, however, is found among the *Artemia* strains studied, *i.e.* from 16 to 34 % for dry weights and from 22 to 37 % for individual caloric contents. No correlation could be found with biotope differences nor with genotypical characteristics. *Artemia* nauplii from the same parental stock (San Francisco Bay, Macau and Barotac Nuevo) metabolize in fact at different rates. A possible source of strain differences might be attributed to the different swimming speeds of the nauplii as reported for at least some of the strains studied here by MILLER *et al.* (1979). The data reported for the different *Artemia* cyst sources provide further evidence for the critical need to standardize the hatching procedures as to always assure feeding with the most energetic form of brine shrimp when using *Artemia* nauplii in fish and crustacean hatcheries (BENIJTS *et al.*, 1976).

Whereas a 24 h aging of the nauplii has a significant impact on their energy content, naupliar viability does not seem to change much after 1 or 2 days of storage. Again significant variation exists among strains. Since COLLINS (1978) observed a correlation between naupliar survival and naupliar size we have performed a detailed correlation analysis between the survival of the nauplii and their various strain characteristics reported in this study. Survival at 20° C and 30° C does not significantly correlate with neither the dry weight nor individual energy content of cysts and instar I nauplii nor with the amount of energy consumed during hatching and early development. Survival at both temperatures, seems, however, to be significantly correlated ($P < 0.05$) with the energy content (expressed in joule/g dry weight) of instar I ($r = 0.55$ - 0.72) and instar II-III nauplii ($r = 0.61$ - 0.64). A significant correlation ($P < 0.05$) also occurs between the mortality rate of the nauplii and the global percentual energy consumption from cyst to instar II-III nauplius ($r = 0.82$ at 20° C ; $r = 0.62$ at 30° C). Although some relationship may exist between the energy content and energy consumption of the nauplii and their survival, the relatively low value for the correlation coefficient suggests that still other parameters may interfere.

ACKNOWLEDGEMENT

This study was supported by the Belgian National Science Foundation (N.F.W.O.) through Grant F.K.F.O. 2.0010.78.

We are very indebted to Dr. ir. E. JASPERS for proofreading the manuscript.

REFERENCES

ABREU-GROBOIS, F. A. and J. A. BEARDMORE (1980) — International Study on *Artemia*. II. Genetic characterization of *Artemia* populations — an electrophoretic approach.

In : The brine shrimp Artemia (Eds G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers). Vol. 1. Morphology, Genetics, Radiobiology, Toxicology, Universa Press, Wetteren (Belgium), 133-146.

BENESCH, R. (1969) — Zur Ontogenie und Morphologie von *Artemia salina* L. *Zool. Jb. Anat.*, **86**, 307-458.

BENIJTS, F., E. VANVOORDEN and P. SORGELOOS (1976) — Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina* L. *In : Proc. 10th European Symposium on Marine Biology* (Eds G. Persoone, E. Jaspers). Vol. 1, Universa Press, Wetteren (Belgium), 1-9.

BOWEN, S. T., J. P. DURKIN, G. STERLING and L. S. CLARK (1978) — *Artemia* hemoglobins : genetic variation in parthenogenetic and zygogenetic populations. *Biol. Bull.*, **155**, 273-287.

BRUGGEMAN, E., P. SORGELOOS and P. VANHAECKE (1980) — Improvements in the decapsulation technique of *Artemia* cysts. *In : The brine shrimp* Artemia (Eds G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers). Vol. 3, Ecology, Culturing, Use in Aquaculture, Universa Press, Wetteren (Belgium), 261-269.

COLLINS, N. C. (1978) — The ecological significance of egg size differences among brine shrimp (*Artemia salina*) populations. *Bull. Ecol. Soc. Am.*, **59** (2), 57.

HAMMER, U. T. (1978) — The saline lakes of Saskatchewan. III. Chemical characterization. *Int. Revue ges. Hydrobiol.*, **63** (3), 311-335.

LITCHFIELD, J. T. (1949) — A method for rapid graphic solution of time-percent effect curves. *J. Pharm. Exp. Ther.*, **97**, 388-408.

LITCHFIELD, J. T. and F. WILCOXON (1949) — A simplified method of evaluating dose-effect experiments. *J. Pharm. Exp. Ther.*, **96**, 99-113.

MACIOLEC, J. A. (1962) — Limnological organic analyses by quantitative dichromatic oxidation. *Fish. Res. Rep.*, **60**, 1-61.

MILLER, D. C., W. H. LANG, M. MARCY, P. CLEM and J. PECHENIK (1979) — International Study on *Artemia*. Naupliar locomotory rates, patterns and photoresponses : A comparative study of various strains of *Artemia salina*. *In : Book of Abstracts. International Symposium on the brine shrimp*, Artemia salina, *Corpus Christi* (*TX-U.S.A.*), Aug. 20-23, 1979; *Artemia* Reference Center, Ghent (Belgium), 91.

PERSOONE, G. and P. SORGELOOS (1972) — An improved separator box for *Artemia* nauplii and other phototactic invertebrates. *Helgoländer wiss. Meeresunters.*, **23**, 243-247.

SNEDECOR, G. W. and W. G. COCHRAN (1967) — *Statistical methods*. The Iowa State University Press, Ames, 593 pp.

SOKAL, R. R. and F. J. ROHLF (1969) — *Biometry*. W. A. Freeman Co., San Francisco, California, 776 pp.

SORGELOOS, P. (1980a) — Improvements in availability and use of *Artemia* as food source for *Macrobrachium*. *In : Giant Prawn* 1980, *Bangkok* (*Thailand*), *June 1980. IFS Provisional Report No 9*. International Foundation for Science, Stockholm (Sweden), 123-132.

SORGELOOS, P. (1980b) — The use of the brine shrimp *Artemia* in aquaculture. *In : The brine shrimp* Artemia (Eds G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers). Vol. 3, Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren (Belgium), 25-46.

SORGELOOS, P., G. PERSOONE, M. BAEZA-MESA, E. BOSSUYT and E. BRUGGEMAN (1978) — The use of *Artemia* cysts in aquaculture : the concept of « hatching efficiency » and description of a new method for cyst processing. *In : Proc. 9th Ann. Meeting WMS.* (Ed. Avault, J. W., jr) Louisiana State University, Baton Rouge (LA-U.S.A.), 715-721.

VANHAECKE, P. and P. SORGELOOS (1980) — International Study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. *In : The brine shrimp* Artemia (Eds G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers). Vol. 3, Ecology Culturing, Use in Aquaculture. Universa Press, Wetteren (Belgium), 393-405.

VANHAECKE, P. and P. SORGELOOS (1983) — International Study on *Artemia*. XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the « hatching efficiency » concept. *Aquaculture*, **30**, 43-52.

VANHAECKE, P., H. STEYAERT and P. SORGELOOS (1980) — International Study on *Artemia*. III. The use of Coulter Counter equipment for the biometrical analysis of *Artemia* cysts. Methodology and mathematics. *In : The brine shrimp* Artemia (Eds G. Persoone, P. Sorgeloos, O. Roels, E. Jaspers). Vol. 1, Morphology, Genetics, Radiobiology, Toxicology. Universa Press, Wetteren (Belgium), 107-115.

VON HENTIG, R. (1971) — Einfluss von Salzgehalt und Temperatur auf Entwicklung, Wachstum, Fortpflanzung und Energiebilanz von *Artemia salina*. *Mar. Biol.*, **9**, 145-182.